

Applicant: Timothy H. Bestor
Serial No.: 09/051,013
Filed: October 9, 1998
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Amendments to the Specification

Please replace the paragraph beginning on page 5, line 10, with the following amended paragraph:

Figure 2. Sequence of HIV-1 5' LTR (SEQ ID NO:1). CpG dinucleotides are bold and underlined; CpG sites that lie within the recognition sequence of methylation-sensitive restriction endonuclease are bold and italicized. Major transcription start site is denoted by +1, and the underlined sequence following denotes the transcribed sequence; numbering is with respect to the transcription start site. Please refer to Figure 1 for the location of protein binding sites. Strike through indicates the 9 bp binding site for the 3 finger protein developed by Wu et al. (1995). Sequence and numbering from Garcia and Gaynor, 1994.

Please replace the paragraph beginning on page 7, line 15, with the following amended paragraph:

Figure 9. Phage-display selection of a zinc finger proteins that bind to predetermined sequences in the HIV-1 5' LTR. The strategy used to construct tridactyl zinc finger proteins that bind to specific sequences was independently developed by the laboratories of Berg (Desjarlais and Berg, 1993), Klug (Choo and Klug, 1994), Pabo (Rebar and Pabo, 1994), and Barbas (Wu et al. 1994). The schemes are very similar; the one depicted here is that of Choo and Klug (1994). The method has been used to obtain proteins that bind to a number of predetermined sequences with high specificity and affinity. The boxed

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sequence shown in the figure appears 20 base pairs 5' of the first HpaII (CCGG) site in the LTR sequence of Figure 2 (SEQ ID NO: 1); it is shown for illustrative purposes only (~~Sequence I.D. No. 2~~). The actual target sequence will be determined as described in Example 2.

Please replace the paragraph beginning on page 9, line 13, with the following amended paragraph:

Figure 14. Targeted methylation demonstrated by bisulfite sequencing analysis. The methylation target shown schematically in Figure 12 was isolated from cells expressing LacI-M.SssI fusion proteins. A portion of the target sequence, including the LacI recognition sequence and three methylation sites is shown at top (SEQ ID NO: 8). The bisulfite sequencing method was used to identify methylated sites. pLM9-1 and -2 are two sister bisulfite clones carrying BS-modified methylation target sequence derived from a pLM9 mutant that has attenuated activity. Methylation is limited to CpG sites in the immediate vicinity of the binding site of the sequence specific DNA binding protein LacI. pBS is the precursor of pLM9 and has no lacI/M.SssI gene; no methylated sites are present. pM is a pBS derivative that encodes fully active (non-targeted) M.SssI gene; all sites are methylated. These data confirm that targeted methylation has been used to direct methylation to CpG sites in the vicinity of the binding site of a sequence-specific DNA binding protein.

Please replace the paragraph beginning on page 9, line 30, with the following amended paragraph:

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Figure 15. Zinc-finger (Zif) targeted methyltransferases.

The above Zif fusion constructs have been made and expression of the appropriate fusion proteins has been confirmed by immunoblot and methylation analysis. The coding regions have also been transferred into the mammalian expression vector pcDNA3.1/His/A. pMZ is represented by SEQ ID NO:2; pZM by SEQ ID NOS: 3-5; pM by SEQ ID NOS:5 and 6; and pZ by SEQ ID NOS:3 and 7.